

## Effects of Elevated Atmospheric Carbon Dioxide on Biomass and Carbon Accumulation in a Model Regenerating Longleaf Pine Community

G. B. Runion,\* M. A. Davis, S. G. Pritchard, S. A. Prior, R. J. Mitchell, H. A. Torbert, H. H. Rogers, and R. R. Dute

### ABSTRACT

Plant species vary in response to atmospheric CO<sub>2</sub> concentration due to differences in physiology, morphology, phenology, and symbiotic relationships. These differences make it very difficult to predict how plant communities will respond to elevated CO<sub>2</sub>. Such information is critical to furthering our understanding of community and ecosystem responses to global climate change. To determine how a simple plant community might respond to elevated CO<sub>2</sub>, a model regenerating longleaf pine community composed of five species was exposed to two CO<sub>2</sub> regimes (ambient, 365  $\mu\text{mol mol}^{-1}$  and elevated, 720  $\mu\text{mol mol}^{-1}$ ) for 3 yr. Total above- and belowground biomass was 70 and 49% greater, respectively, in CO<sub>2</sub>-enriched plots. Carbon (C) content followed a response pattern similar to biomass, resulting in a significant increase of 13.8 Mg C ha<sup>-1</sup> under elevated CO<sub>2</sub>. Responses of individual species, however, varied. Longleaf pine (*Pinus palustris* Mill.) was primarily responsible for the positive response to CO<sub>2</sub> enrichment. Wiregrass (*Aristida stricta* Michx.), rattlebox (*Crotalaria rotundifolia* Walt. Ex Gmel.), and butterfly weed (*Asclepias tuberosa* L.) exhibited negative above- and belowground biomass responses to elevated CO<sub>2</sub>, while sand post oak (*Quercus margaretta* Ashe) did not differ significantly between CO<sub>2</sub> treatments. As with pine, C content followed patterns similar to biomass. Elevated CO<sub>2</sub> resulted in alterations in community structure. Longleaf pine comprised 88% of total biomass in CO<sub>2</sub>-enriched plots, but only 76% in ambient plots. In contrast, wiregrass, rattlebox, and butterfly weed comprised 19% in ambient CO<sub>2</sub> plots, but only 8% under high CO<sub>2</sub>. Therefore, while longleaf pine may perform well in a high CO<sub>2</sub> world, other members of this community may not compete as well, which could alter community function. Effects of elevated CO<sub>2</sub> on plant communities are complex, dynamic, and difficult to predict, clearly demonstrating the need for more research in this important area of global change science.

ATMOSPHERIC CO<sub>2</sub> concentration is rising, due primarily to fossil fuel combustion and deforestation, and is projected to double preindustrial levels within the next century (Keeling and Whorf, 1994). Experimentally doubling atmospheric CO<sub>2</sub> has been shown to in-

crease plant biomass, on average, by almost 40% (Poorter, 1993). However, all species do not exhibit equivalent responses to CO<sub>2</sub> enrichment. Leaf morphology (Ceulemans and Mousseau, 1994; Saxe et al., 1998), physiology (Drake, 1992; Poorter, 1993), symbiotic relationships (Hartwig et al., 1996), and competition (Bazzaz and Carlson, 1984; Pritchard et al., 2001; Davis et al., 2002) all influence plant response to CO<sub>2</sub>.

While individual plant response to elevated CO<sub>2</sub> may be predicted based on differences in the factors noted above, evidence from competition studies consistently suggests that response cannot be reliably predicted when species are grown in communities (Bazzaz and Carlson, 1984; Bazzaz, 1990; Bazzaz and McConnaughay, 1992; Ziska, 2003; Morgan et al., 2004). Given that CO<sub>2</sub>-induced shifts in competitive advantages among species may alter species composition and community structure and function (Wray and Strain, 1987; Fajer, 1989; Bazzaz, 1990; Joel et al., 2001; Dijkstra et al., 2002), experiments which examine the effects of CO<sub>2</sub> enrichment on plant communities are critical for furthering our understanding of ecosystem response to global climate change.

Study of elevated CO<sub>2</sub> effects on plants under competition has been a topic for investigation for over two decades (see review by Bazzaz, 1990). Initial work tended to concentrate on annual plant species grown for short durations in containers placed in glasshouses or growth chambers (Bazzaz and Carlson, 1984; Reekie and Bazzaz, 1989; Körner and Arnone, 1992). This work was expanded to open-top chambers, but continued to be conducted using containers and for relatively short durations. More recent work has focused on field-grown plants utilizing open-top chambers and FACE (free-air CO<sub>2</sub> enrichment). However, an extreme paucity of information still exists for forest ecosystems, particularly tropical seasonal forests and dry tropical communities such as savannas and scrub vegetation ecosystems (Arnone, 1996), and the need for studies of forests and other long-lived plant species exposed for longer durations continues to be noted (Norby et al., 1999; Ward and Strain, 1999).

Before European settlement, longleaf pine savannas occupied 37.2 million ha of the southeastern United States (Landers et al., 1995). Since the early 1600s, timber harvesting, fire suppression, and conversion of forests to farmland have reduced the land area of these ecosystems to less than 4% of their original range (Peet and Allard, 1993; Landers et al., 1995). This loss is comparable to that of many endangered communities including the North American tallgrass prairie, the moist tropical coastal forest of Brazil, and the dry forests along the Pacific coast of Central America (Noss, 1989). Longleaf pine ecosystems are also very diverse,

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with some specific types having the highest reported values for species richness, including many threatened and endangered species, in the temperate Western Hemisphere (e.g., 140 species ha<sup>-1</sup> for Mesic Longleaf Woodlands; Peet and Allard, 1993). To protect and maintain these important longleaf pine ecosystems it is necessary to understand the impacts of global change, including response to CO<sub>2</sub> enrichment, on their structure and function.

Davis et al. (2002) reported results from a model regenerating longleaf pine community, composed of species representing different structural and functional guilds, following 2 yr of exposure to ambient and elevated levels of atmospheric CO<sub>2</sub>. They reported that total biomass for this community increased by 109% due primarily to increases in longleaf pine (117%) and in wiregrass (24%), the other keystone species of this community. The three other species in this study (sand post oak, rattlebox, and butterfly weed) showed either no effect or a decline in biomass (directly and as a percentage of total community biomass) when grown under high CO<sub>2</sub>. It should be noted that the aboveground results of Davis et al. (2002) were derived primarily from allometric equations. Similarly, belowground results from this study (Pritchard et al., 2001) were also derived using nondestructive minirhizotron technology.

Here we report above- and belowground biomass and carbon content determined following a complete destructive harvest and following an additional year of exposure to ambient and elevated atmospheric CO<sub>2</sub> for this model regenerating longleaf pine community. Specific hypotheses addressed by this study were similar to those reported by Davis et al. (2002) and were as follows: (i) C<sub>3</sub> plants would increase growth to a greater extent than C<sub>4</sub> plants due to their greater photosynthetic response to elevated CO<sub>2</sub> (Bowes, 1993; Amthor, 1995); (ii) broadleaf C<sub>3</sub> plants would respond more favorably to elevated CO<sub>2</sub> than conifers due to being anatomically and physiologically better adapted to assimilate extra carbon (Ceulemans and Mousseau, 1994; Pritchard et al., 1998); (iii) N-fixing plant response to elevated CO<sub>2</sub> would exceed that of non-fixers; and (iv) the influence of elevated CO<sub>2</sub> on competitive relationships, acting through the mechanisms elucidated above, would alter structure of this forest community and its capacity for storing atmospheric carbon.

## MATERIALS AND METHODS

### Study Site

A model regenerating longleaf pine–wiregrass community was constructed in spring 1998 at the soil bin facilities of the National Soil Dynamics Laboratory in Auburn, AL. This model community was used to examine effects of CO<sub>2</sub> enrichment on plants at multiple scales, including leaf, whole-plant, and community. Descriptions of the study site and model community have been previously reported (Pritchard et al., 2001; Davis et al., 2002). Briefly, an assemblage of five early successional forest species representing major functional guilds within a typical longleaf pine–wiregrass community were chosen for study: longleaf pine, a C<sub>3</sub> evergreen conifer; wiregrass, a C<sub>4</sub> bunch grass; sand post oak, a C<sub>3</sub> broadleaf tree;

rattlebox, a C<sub>3</sub> perennial, herbaceous, N-fixing legume; and butterfly weed, a C<sub>3</sub>, non-leguminous, herbaceous perennial. These species are common associates in early successional longleaf pine savannahs throughout the southeastern United States (Abrahamson and Hartnett, 1990). Before transplanting into the model community, all plants were grown in 15-cm<sup>3</sup> containers from seed collected from natural sources.

This forest community was assembled in April 1998 on an outdoor soil bin (2 m deep, 6 m wide, and 76 m long) containing a Blanton loamy sand (loamy, siliceous, thermic Grossarenic Paleudults) taken from a longleaf pine area (Sandhills or Subseric Gulf Coastal Plain type; *sensu* Peet and Allard, 1993) typical of the Southeastern Coastal Plains. Before planting, the soil bin was divided into 0.75-m<sup>2</sup> quadrats each possessing 16 equally spaced planting positions. The community was constructed by randomly assigning individuals of each species (three longleaf pine, three wiregrass, two sand post oak, one rattlebox, and one butterfly weed) into positions within each quadrat; six planting spaces per quadrat were left empty. This regime achieved planting densities reflective of naturally regenerating longleaf pine–wiregrass ecosystems (Hains, 1995; Jacqumain, 1996). Plants were regularly irrigated during summer 1998 to facilitate community establishment using a metered drip irrigation system to deliver exact and consistent watering throughout the bin; thereafter, plants received only ambient rainfall. During the first 2 mo dead plants were replaced; thereafter, mortality was attributed to causes other than transplanting, and gaps were not refilled.

Open-top chambers (Rogers et al., 1983), encompassing 7.3 m<sup>2</sup> of ground surface area, were used to deliver target CO<sub>2</sub> concentrations of 365 μmol mol<sup>-1</sup> (ambient) or 720 μmol mol<sup>-1</sup> (elevated) beginning June 1998 using a delivery system described by Mitchell et al. (1995). Actual CO<sub>2</sub> concentrations over the 3-yr measurement period (±SE) were as follows: ambient daytime = 376.2 (±0.1); elevated daytime = 701.4 (±0.2); ambient nighttime = 407.6 (±0.01); and elevated nighttime = 758.6 (±0.2) (daytime was taken as 0700 to 1900 h CST; *n* was approximately 91 000 for each measurement). The bin was divided into six blocks and each CO<sub>2</sub> treatment was randomly assigned to one open top chamber within each block. The experimental design was a randomized complete block design, with blocks occurring along the length of the soil bin.

### Aboveground Biomass

Aboveground portions of plants in experimental plots were destructively harvested in June 2001 after 3 yr of CO<sub>2</sub> exposure. All plants within a plot were harvested in the order of: longleaf pine, sand post oak, butterfly weed, rattlebox, and wiregrass. Following harvest, aboveground parameters (e.g., height, diameter, numbers of branches and/or leaves) were assessed using standard practices. Diameters were measured at ground line using high precision digital calipers. Individual plants were then subdivided into component organs depending on species: longleaf pine and sand post oak were separated by leaves, branches, and stems; butterfly weed plants were separated into leaves and stems (branches were included in stem biomass); the morphology of rattlebox and wiregrass did not facilitate the separation of leaves from stems. Plant component parts were placed in paper bags and dried to a constant weight at 55°C in a forced-air drying oven. Following removal of aboveground biomass, all litter was removed from plots, separated by species, oven-dried, and weighed. Following drying, biomass components and litter were ground separately to pass through a 0.2-mm mesh sieve. Carbon and nitrogen concentrations were determined using a LECO (St. Joseph, MI) 600-CHN analyzer.

### Belowground Biomass

Immediately following harvest of aboveground biomass, fine root biomass was assessed using large soil cores (24.5 cm diameter  $\times$  60 cm deep) and an extraction method of our own design (Prior et al., 2004). Steel core tubes were driven into the ground using a hydraulic cylinder mounted on the front of a small tractor and an iron driving head which rested on the outer top edge of the steel core tube. Once the tube had been driven completely into the ground (i.e., so the top of the tube was even with the soil surface), the driving head was removed and the hydraulic cylinder was connected to a chain attached to a collar located just below a small outer lip of the core tube. The hydraulic cylinder was then used to remove the core tube with soil from the ground. Eight cores were extracted from each plot. Extracted core tubes were immediately placed into buckets (to prevent loss of soil from the bottom of the tube) and moved to a hydraulic device which pushed the soil out of the core tube to a set distance (i.e., 15 cm) and this quantity of soil was sliced from the main tube. This process was repeated four times for each core tube, giving core depth increments of 0 to 15, 15 to 30, 30 to 45, and 45 to 60 cm. All soil from each depth increment was sieved through a 2-mm mesh screen; roots were removed, bagged, and stored in a walk-in cold room (4°C) until processing. Fine roots were separated, based on color and morphology, into three classes (longleaf pine, sand post oak, and other). The latter class of fine roots was most likely dominated by wiregrass, which has a fibrous root system. Rattlebox and butterfly weed have tuberous root systems with limited lateral root spread and the vast majority of their roots were obtained during the spade extraction. Each class of roots was further segregated into three diameter classes ( $>2$ ,  $0.5$ – $2$ , and  $<0.5$  mm) before placement in a drying oven for dry weight determination, as previously described. As with aboveground biomass, all root samples were ground and carbon and nitrogen concentrations determined.

Following coring, coarse root biomass was assessed using one of two methods. Coarse roots for wiregrass, rattlebox, and butterfly weed were dug from each plot using standard sharp-shooter spades. Two spades (one on each side of the plant) were inserted into the soil about 25 cm away from the plant to a depth of about 30 cm, the soil was loosened, and the entire root system retrieved. Coarse roots for longleaf pine and sand post oak were extracted by: removing a small volume of soil from around each stump by hand; connecting a clamp to the stump just below the soil surface; connecting the clamp to a hydraulic cylinder mounted on the front of a small tractor; and raising the cylinder until the taproot and attached large lateral roots were loosened from the soil. After soaking in water, coarse roots were washed free of soil using a soft bristle brush, placed in paper bags, and dried to a constant weight at 55°C. Undoubtedly, the extraction of longleaf pine and sand post oak did not recover the entire coarse root biomass for each tree; however, as the same technique was employed for all trees in all plots, the relative amount of coarse roots removed should be comparable.

### Data Analysis

Data analysis was conducted using the mixed model procedures (Proc Mixed) of the Statistical Analysis System (Littell et al., 1996). Error terms appropriate to the randomized block design were used to test the significance of CO<sub>2</sub> concentration. In all cases, differences were considered significant at the  $\alpha \leq 0.05$  and trends were recognized at  $0.05 \leq \alpha \leq 0.15$ .

## RESULTS

### Species Response

#### Longleaf Pine

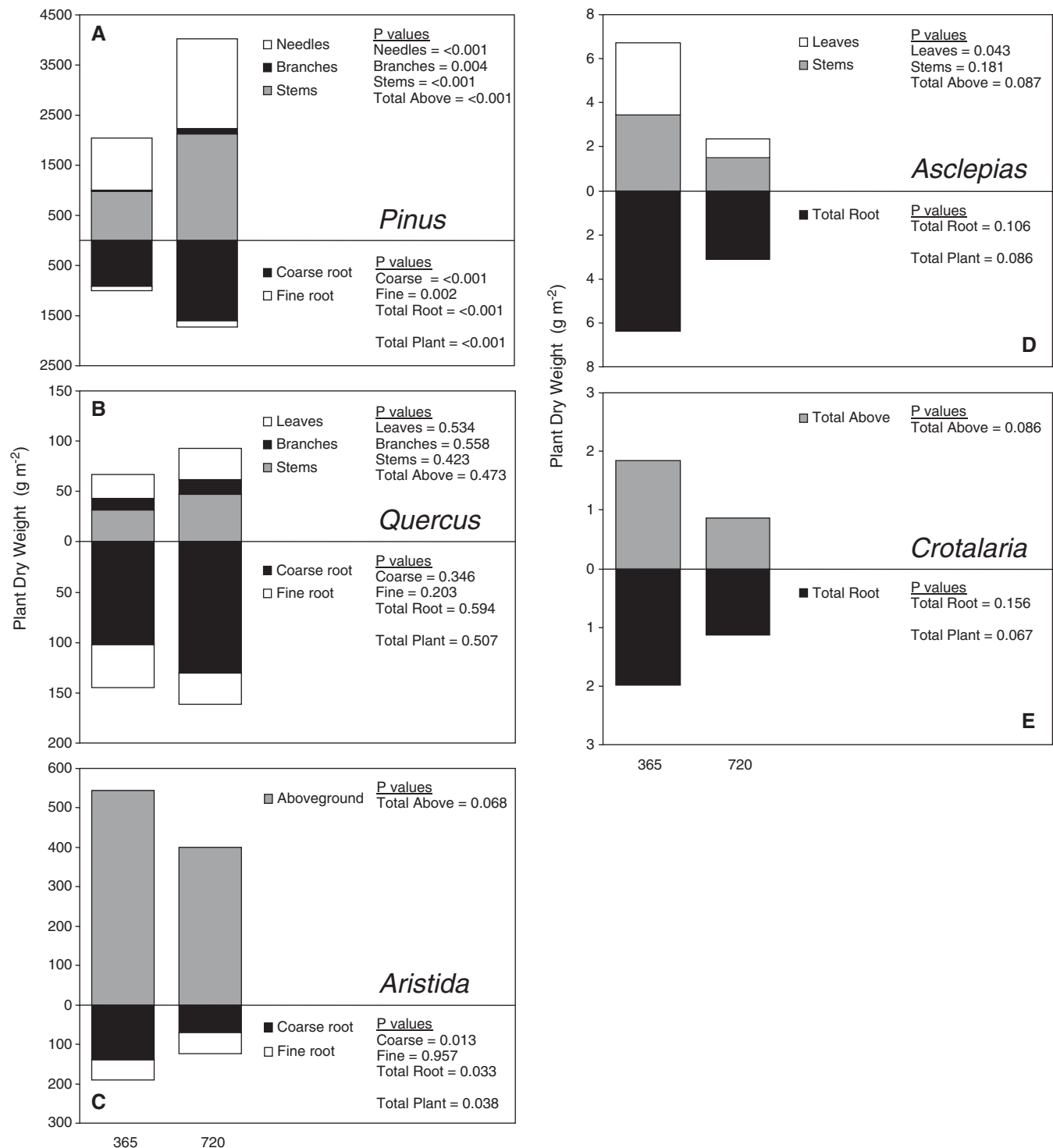
Percent mortality did not differ between CO<sub>2</sub> treatments. However, all anatomical measurements (i.e., height, diameter, and branch number, length, and density) were significantly greater for plants grown under elevated CO<sub>2</sub> (Table 1). Concomitant with this increase in growth, dry weight of all plant organs (i.e., needles, branches, stems, coarse roots, and fine roots) were significantly greater when grown under high CO<sub>2</sub> (Fig. 1A), resulting in an 88% increase in total plant dry weight. Dry weight of plant litter was also significantly higher for plants grown under high, compared with ambient, CO<sub>2</sub> (Table 1). Elevated atmospheric CO<sub>2</sub> resulted in changes in biomass allocation patterns (Fig. 2A). Allocation to branches and stems was increased under high CO<sub>2</sub>, while allocation to needles and roots was decreased. This altered allocation resulted in a significantly lower root to shoot ratio for plants grown under elevated CO<sub>2</sub> (Fig. 3).

#### Sand Post Oak

No significant differences between CO<sub>2</sub> treatments were detected for any anatomical (Table 1) or biomass

**Table 1.** The response of growth and litter variables to ambient (365  $\mu\text{mol mol}^{-1}$ ) and elevated (720  $\mu\text{mol mol}^{-1}$ ) CO<sub>2</sub> for the five species following 3 yr of exposure. Means with associated separation statistics and percent change (ambient to elevated) are shown.

Parameter	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	Percent change	P values
<b>Longleaf pine</b>				
Mortality, %	16.5	14.5	–12.1	0.604
Height, cm	109.3	159.1	45.6	<0.001
Diameter, cm	4.20	4.74	12.9	<0.001
Branch number	1.9	3.7	94.7	0.002
Branch density, no. m <sup>-1</sup>	1.3	1.9	46.2	0.030
Average branch length, cm	4.9	10.4	112.2	0.001
Litter dry weight, g m <sup>-2</sup>	387.6	684.2	76.5	<0.001
<b>Sand post oak</b>				
Mortality, %	10.8	7.8	–27.8	0.434
Height, cm	43.7	54.9	25.6	0.264
Leaf number	52.6	64.3	22.2	0.562
Leaf density, no. m <sup>-1</sup>	89.4	83.1	–7.0	0.508
Litter dry weight, g m <sup>-2</sup>	10.1	17.3	71.3	0.185
<b>Wiregrass</b>				
Mortality, %	9.7	19.1	96.9	0.009
Diameter, cm	5.23	5.02	–4.0	0.330
Total clump area, cm <sup>2</sup>	913.0	692.4	–24.2	0.009
Litter dry weight, g m <sup>-2</sup>	25.3	60.2	137.9	0.012
<b>Butterfly weed</b>				
Mortality, %	61.6	69.3	12.5	0.504
Height, cm	73.7	58.4	–20.8	0.261
Stem length, cm	179.8	95.8	–46.7	0.025
Reproductive plants, %	53.1	33.1	–37.7	0.245
Litter dry weight, g m <sup>-2</sup>	22.2	20.2	–9.0	0.783
<b>Rattlebox</b>				
Mortality, %	–4.4	13.1	–	0.514
Reproductive plants, %	30.2	22.1	–26.8	0.242
Litter dry weight, g m <sup>-2</sup>	11.5	23.3	102.6	0.269



**Fig. 1.** Plant dry weight ( $\text{g m}^{-2}$ ) for component plant parts (needles, leaves, branches, stems, total aboveground, coarse roots, fine roots, total roots, total plant as applicable for each species), with associated mean separation statistics, for longleaf pine (A), sand post oak (B), wiregrass (C), butterfly weed (D), and rattlebox (E) when grown together for 3 yr under ambient ( $365 \mu\text{mol mol}^{-1}$ ) and elevated ( $720 \mu\text{mol mol}^{-1}$ ) concentrations of atmospheric CO<sub>2</sub>.

(Fig. 1B) parameters. There was, however, a decrease in biomass allocation to fine roots (Fig. 2B), which resulted in a trend for decreased root to shoot ratio under elevated CO<sub>2</sub> (Fig. 3).

### Wiregrass

Percent mortality was significantly higher under elevated than ambient CO<sub>2</sub> (Table 1). Clump diameter of wiregrass was not affected by CO<sub>2</sub> treatment. However,



**Table 2. Biomass allocation among species when grown for 3 yr under ambient ( $365 \mu\text{mol mol}^{-1}$ ) and elevated ( $720 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$ . Means with associated separation statistics and percent change (ambient to elevated) are shown.**

Percent of total in species	Ambient $\text{CO}_2$	Elevated $\text{CO}_2$	Percent change	<i>P</i> values
% Longleaf pine				
Aboveground	76.74	88.86	15.8	<0.001
Belowground	74.04	85.46	15.4	<0.001
Total	75.81	87.81	15.8	<0.001
Sand post oak				
Aboveground	2.50	2.06	−17.6	0.688
Belowground	10.55	8.06	−23.6	0.153
Total	5.21	3.92	−24.8	0.283
Wiregrass				
Aboveground	20.44	9.01	−55.9	0.003
Belowground	14.81	6.28	−57.6	0.001
Total	18.57	8.16	−56.1	0.004
Butterfly weed				
Aboveground	0.25	0.05	−80.0	0.054
Belowground	0.46	0.15	−67.4	0.016
Total	0.32	0.08	−75.0	0.021
Rattlebox				
Aboveground	0.07	0.02	−71.4	0.041
Belowground	0.14	0.05	−64.3	0.033
Total	0.09	0.03	−66.7	0.019

total clump area was 24% lower under elevated  $\text{CO}_2$  conditions (Table 1) due to increased mortality. Biomass of plant organs, with the exception of fine roots, was lower under  $\text{CO}_2$ -enriched conditions (Fig. 1C), resulting in 29% less total plant dry weight. However, litter was significantly increased by growth under elevated  $\text{CO}_2$  (Table 1). Elevated atmospheric  $\text{CO}_2$  tended to decrease biomass allocation to coarse roots, but increased allocation to fine roots (Fig. 2C); this resulted, overall, in little net change and no effect on root to shoot ratio (Fig. 3).

### Butterfly Weed

Elevated  $\text{CO}_2$  did not affect percent mortality, number of reproductive plants, litter dry weight, or height. However, total stem length (cumulative for all stems) was lower for plants growing under high  $\text{CO}_2$  (Table 1). Biomass of all plant organs was (or tended to be) lower under  $\text{CO}_2$ -enriched conditions, resulting in elevated  $\text{CO}_2$  plots having less than half as much biomass both above- and belowground (Fig. 1D). Elevated  $\text{CO}_2$  decreased biomass allocation to leaves, but increased allocation to roots (Fig. 2D); this resulted in an increase in root to shoot ratio (Fig. 3).

### Rattlebox

As with butterfly weed, elevated  $\text{CO}_2$  did not affect percent mortality, number of reproductive plants, or litter dry weight (Table 1). Biomass of both above- and belowground plant parts tended to be lower under  $\text{CO}_2$ -enriched conditions. As with butterfly weed, this resulted in elevated  $\text{CO}_2$  plots having approximately half as much biomass as ambient plots (Fig. 1E). However, unlike butterfly weed, elevated  $\text{CO}_2$  did not af-

fect biomass allocation (Fig. 2E) nor root to shoot ratio (Fig. 3).

## Community Level Responses

The total aboveground biomass of  $\text{CO}_2$ -enriched plots was 70% greater than that of ambient plots ( $P < 0.001$ ). Increases in woody biomass (stems and branches) were responsible for 67% of the difference in aboveground response to  $\text{CO}_2$ . Belowground biomass was also greatly enhanced by elevated  $\text{CO}_2$  (+41%,  $P < 0.001$ ). Although fine root biomass was significantly greater in  $\text{CO}_2$ -enriched plots (+10%,  $P = 0.007$ ), increases in coarse root biomass were responsible for 97% of the belowground response to elevated  $\text{CO}_2$ . Total leaf litter mass was 76% greater in  $\text{CO}_2$ -enriched plots ( $P < 0.001$ ).

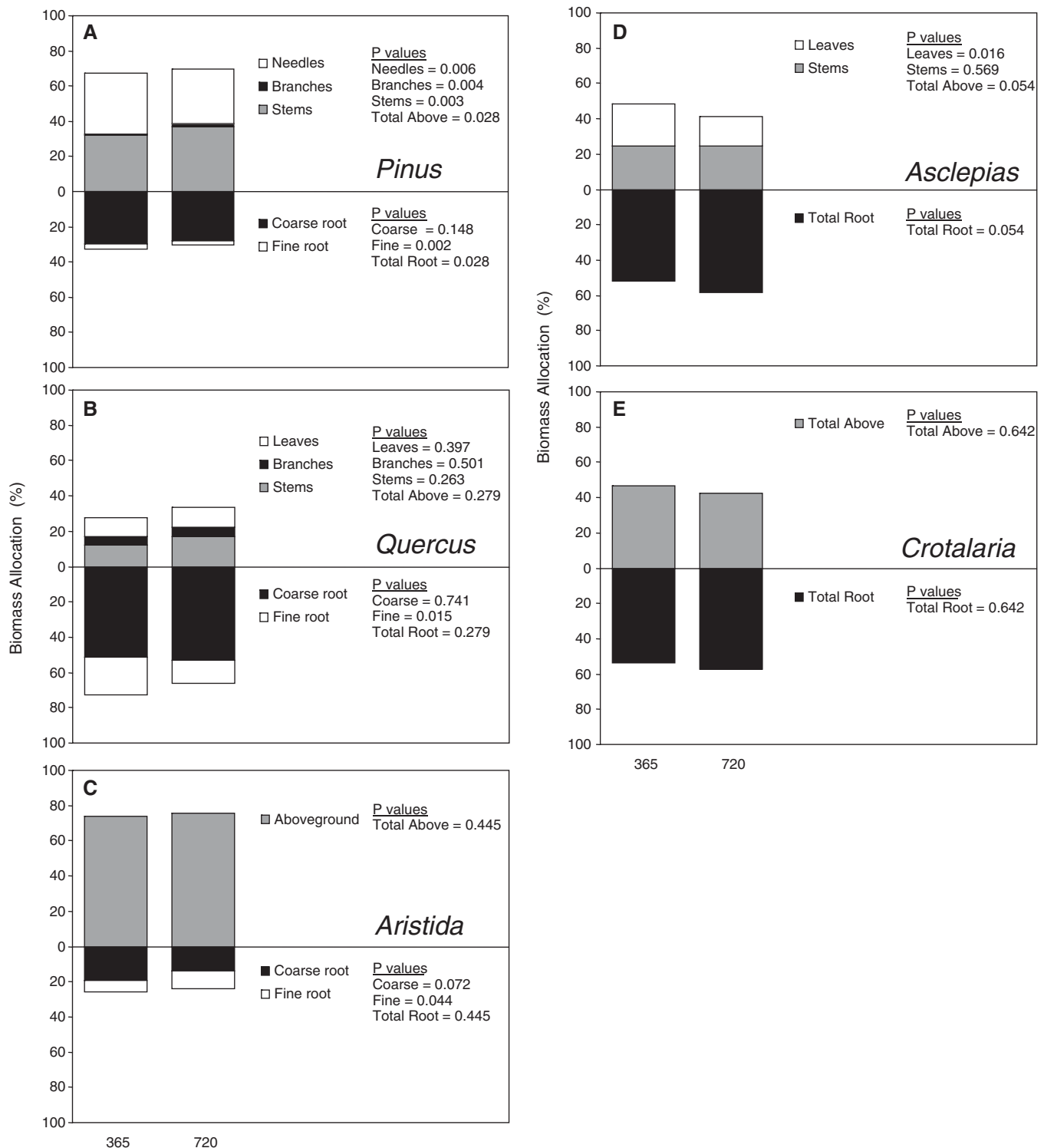
Community structure was altered by  $\text{CO}_2$  enrichment. The percentage of aboveground, belowground, and total biomass comprised by longleaf pine was significantly greater (approximately 12%) in  $\text{CO}_2$ -enriched plots (Table 2). Biomass components for wiregrass were, concomitantly, lower (8–11%) under elevated  $\text{CO}_2$  conditions. Although butterfly weed and rattlebox comprised small amounts of the overall biomass of the system, these were also significantly lower when grown under elevated than ambient  $\text{CO}_2$  (Table 2). As with most other variables, the biomass contribution of sand post oak to the total system did not differ significantly between  $\text{CO}_2$  treatments.

## Carbon Responses

Carbon concentration ( $\text{mg g}^{-1}$ ) of longleaf pine stems ( $P = 0.002$ ) and fine roots ( $P = 0.106$ ) tended to be higher, while that of longleaf pine litter ( $P = 0.011$ ), sand post oak coarse roots ( $P = 0.015$ ), and butterfly weed leaves ( $P = 0.004$ ) were lower under elevated  $\text{CO}_2$  (data not shown). Carbon concentration of all other plant tissues, as well as that for soil, was not affected by  $\text{CO}_2$  treatment. Carbon content ( $\text{g m}^{-2}$ ) of plant tissues and litter (data not shown) followed patterns, with regard to differences between  $\text{CO}_2$  treatments and their statistical significance, exactly in accordance with tissue dry weight data (Table 1 and Fig. 1). Within this model community, total C content ( $\text{g m}^{-2}$ ) of plants and litter were significantly increased (65 and 74%, respectively) by 3 yr of growth under elevated  $\text{CO}_2$  (Table 3). Soil C content did not differ between  $\text{CO}_2$  treatments at termination of this study. However, as soil C content was slightly higher in elevated  $\text{CO}_2$  plots at initiation of the study, the overall gain in soil C was lower under elevated  $\text{CO}_2$  (Table 3). Nonetheless, due to the large gain in C content in both standing plant and litter biomass, total C content and total C gain over the course of study were significantly increased by exposure to elevated atmospheric  $\text{CO}_2$  (Table 3).

## DISCUSSION

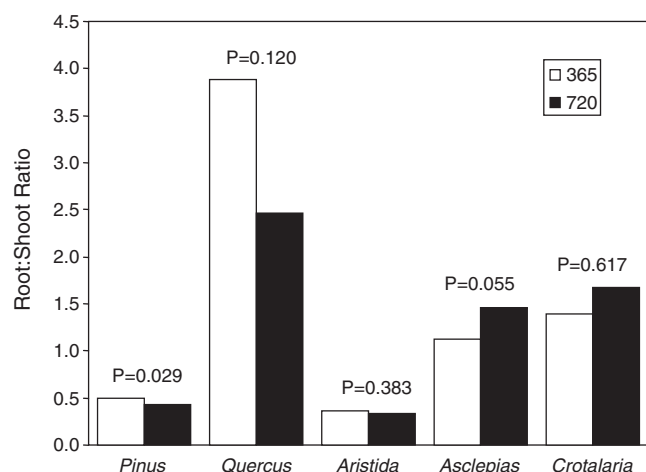
While much is known concerning the response of individual plants to increasing atmospheric  $\text{CO}_2$  con-



**Fig. 2.** Biomass allocation (%) among plant component parts (needles, leaves, branches, stems, total aboveground, coarse roots, fine roots, total roots, total plant as applicable for each species), with associated mean separation statistics, for longleaf pine (A), sand post oak (B), wiregrass (C), butterfly weed (D), and rattlebox (E) when grown together for 3 yr under ambient (365  $\mu\text{mol mol}^{-1}$ ) and elevated (720  $\mu\text{mol mol}^{-1}$ ) concentrations of atmospheric CO<sub>2</sub>.

centration (Amthor, 1995), few experiments have addressed community level responses to elevated CO<sub>2</sub>; this paucity of data is particularly severe for forest ecosystems (Arnold, 1996). Forests are both ecologically and economically important in that they contribute as much

as two-thirds to the world's photosynthesis (Kramer, 1981), they dominate many terrestrial ecosystems, and they provide raw materials for a multi-billion dollar industry each year. Thus, understanding how global change (including the effects of elevated CO<sub>2</sub>) will



**Fig. 3.** Root to shoot ratios, with associated mean separation statistics, for longleaf pine, sand post oak, wiregrass, butterfly weed, and rattlebox when grown together for 3 yr under ambient ( $365 \mu\text{mol mol}^{-1}$ ) and elevated ( $720 \mu\text{mol mol}^{-1}$ ) concentrations of atmospheric  $\text{CO}_2$ .

influence the structure and function of forest communities is critical to accurately predict how the biosphere may be impacted.

Integrating knowledge from the  $\text{CO}_2$  effects literature (particularly physiology, symbiotic relationships, and nutrient acquisition of individual plants) with our prior research on the effects of  $\text{CO}_2$  on longleaf pine (Prior et al., 1997; Runion et al., 1997, 1999), this research was developed to test four hypotheses concerning how plants from differing functional guilds would respond to elevated  $\text{CO}_2$  when grown in a community. Briefly, we predicted  $\text{C}_3 > \text{C}_4$  plants, broadleaf  $>$  conifers, N-fixers  $>$  non-N-fixers, and that the influence of elevated  $\text{CO}_2$  on these competitive relationships would alter structure of this forest community and its capacity for storing atmospheric carbon.

Growth responses from the destructive harvest reported here (3 yr) are in general agreement with a previous report from this study which used allometric equations to estimate biomass following 2 yr of exposure to elevated and ambient  $\text{CO}_2$  (Davis et al., 2002). Aboveground community biomass was estimated to be 109% greater in response to  $\text{CO}_2$  enrichment (compared with 70% reported here based on actual harvested material). One major difference between the previous report and data presented here was the response of wiregrass to the  $\text{CO}_2$  treatments. After 2 yr, wiregrass aboveground biomass was 24% greater in elevated than ambient  $\text{CO}_2$  plots (Davis et al., 2002). However, after the third year, wiregrass aboveground biomass was actually 26% less in  $\text{CO}_2$ -enriched chambers. This difference in wiregrass biomass response to  $\text{CO}_2$  was primarily due to the increased mortality which occurred between the two reporting periods (i.e., 5% for both treatments after 2 yr compared with 9.7% and 19.1% for ambient and elevated  $\text{CO}_2$  treatments after 3 yr) which is most likely due to shading effects of longleaf pine.

Data from this study support our first hypothesis in that biomass of  $\text{C}_3$  plants, as a whole, responded posi-

**Table 3.** Carbon content of various longleaf pine community components when grown for 3 yr under ambient ( $365 \mu\text{mol mol}^{-1}$ ) and elevated ( $720 \mu\text{mol mol}^{-1}$ )  $\text{CO}_2$ . Means with associated separation statistics and percent change (ambient to elevated) are shown.

Component	Ambient $\text{CO}_2$	Elevated $\text{CO}_2$	Percent change	P values
	$\text{g m}^{-2}$			
Total plants	1886.2	3106.7	64.7	<0.001
Total litter	217.1	378.3	74.3	<0.001
Initial soil	1764.3	1942.0	10.1	0.097
Final soil	3042.3	2974.7	-2.2	0.281
Gain in soil	1278.0	1032.7	-19.2	0.040
Total	5145.5	6459.6	25.5	<0.001
Total gain	3381.3	4517.6	33.6	<0.001

tively to elevated  $\text{CO}_2$ , while survival and growth of the  $\text{C}_4$  species (wiregrass) was reduced. Unexpectedly, and counter to our second hypothesis, increased atmospheric  $\text{CO}_2$  strongly favored the  $\text{C}_3$  conifer (longleaf pine) over the broadleaf  $\text{C}_3$  species (sand post oak, rattlebox, and butterfly weed). Longleaf pine begins in a grass stage, where it remains for 3 to 6 (even up to 12) years (Harlow and Harrar, 1969). Once out of the grass stage, longleaf pine generally grows very rapidly due to its extensive tap root system developed during the grass stage. Plants grown under elevated  $\text{CO}_2$  tended to bolt out of the grass stage earlier than those in ambient plots and were 30 cm taller 2 yr after planting (Davis et al., 2002). By the end of the third year the increase from high  $\text{CO}_2$  was almost 50 cm. This early and rapid height growth under high  $\text{CO}_2$  is the most likely reason for the poorer performance of the other species (including the  $\text{C}_3$  broadleaves) in this study (i.e., other plants did not put on rapid height growth, could not compete for light, and succumbed to shading by longleaf pine).

A previous study (Runion et al., 1997) demonstrated that, due both to increased root length and increased colonization, ectomycorrhizae approximately doubled on longleaf pine exposed to elevated  $\text{CO}_2$ . Further, it has been suggested that vesicular-arbuscular mycorrhizae (which occur on grasses, legumes, and forbes) tend to be less responsive to elevated  $\text{CO}_2$  than do ectomycorrhizae (O'Neill, 1994). Although not measured in this study, increased ectomycorrhizal colonization might also help explain the strong response of longleaf pine to elevated  $\text{CO}_2$  (compared with other  $\text{C}_3$  species). However, this explanation would not hold for sand post oak, which also has ectomycorrhizal associations, as it showed no significant response to high  $\text{CO}_2$ . It should be noted that longleaf pine, as an evergreen conifer, is capable of photosynthesis and root growth (thus, exploration for soil resources) during the overwintering period giving it a possible competitive advantage over neighboring deciduous species, regardless of any potential impacts on mycorrhizal associations.

Our third hypothesis is also not supported by data from this study. Rattlebox, the  $\text{C}_3$  N-fixing legume in this study, did not respond positively to elevated  $\text{CO}_2$  and did not perform any better than butterfly weed (a similar, albeit non-N-fixing,  $\text{C}_3$  herbaceous perennial) or wiregrass (a  $\text{C}_4$  grass). The fact that tissue N concentration was unaffected by  $\text{CO}_2$  treatment for all species (data not

shown), suggests that plant growth in this study was not limited by soil N availability. Therefore, the ability to fix N did not provide rattlebox with any substantial benefit over the other understory species and it, too, was negatively affected by shading from longleaf pine. Grasses, such as wiregrass, have been shown to compete strongly for soil resources, including N (Mitchell et al., 1993; Perry et al., 1993, 1994). Thus, it is possible that wiregrass may have moderated response of the C<sub>3</sub> understory species (rattlebox, butterfly weed, and sand post oak) to elevated CO<sub>2</sub>. Given the large growth response of longleaf pine, it appears likely that it did not suffer from competition with wiregrass for soil resources.

In both the initial report (Davis et al., 2002) and here, community structure was altered by CO<sub>2</sub> enrichment (Hypothesis 4). However, it is important to note that shifts in individual species responses occurred between these two reports, with the most notable changes observed for the keystone species, longleaf pine and wiregrass. Previously, the percentage of aboveground biomass comprised by longleaf pine was only 4% greater in CO<sub>2</sub>-enriched plots (Davis et al., 2002), while at the destructive harvest 1 yr later this difference was increased threefold (approximately 12%). After 2 yr, wiregrass comprised only 2% less of the total system biomass in CO<sub>2</sub>-enriched chambers (Davis et al., 2002). After the third year, the contribution of wiregrass to total system biomass was greatly decreased (11%) under high CO<sub>2</sub> conditions. This difference in wiregrass response to CO<sub>2</sub> is primarily due to the increased mortality which occurred between the two reporting periods (i.e., 5% for both treatments after 2 yr compared with 9.7 and 19.1% for ambient and elevated CO<sub>2</sub> treatments after 3 yr) which is most likely due to shading effects of longleaf pine.

The rapid growth of longleaf pine, particularly in height, under high CO<sub>2</sub> likely affected not only wiregrass mortality, but the response of the other understory species. Since longleaf pine was one of the more densely planted species, its rapid height growth under elevated CO<sub>2</sub> tended to produce a greater shading effect (Davis et al., 2002), which likely dampened the response of understory species. Regardless of the cause(s), elevated CO<sub>2</sub> did alter species composition in this study. Davis et al. (2002) suggested that the CO<sub>2</sub>-induced changes in community structure noted after 2 yr would probably not alter community function as both of the keystone species (longleaf pine and wiregrass) performed well under high CO<sub>2</sub>. Results from this third year clearly demonstrate that the performance of wiregrass under elevated CO<sub>2</sub> was not sustainable and suggest that the effects on community function could be dramatic. For example, longleaf pine-wiregrass ecosystems are fire-dependent and fire is critical in nutrient cycling in these systems. The primary pyric species in this system are longleaf pine and wiregrass. Thus, the pyric characteristics of these ecosystems may lessen as CO<sub>2</sub> concentration continues to rise, given the poor response of wiregrass to elevated CO<sub>2</sub>, unless it is replaced by another pyric understory grass species. On the other hand, given the increased litter production noted here, fire intensity in these systems may increase or fire manage-

ment may need to be altered (increased frequency) as CO<sub>2</sub> continues to rise.

The apparent increased competitive ability of longleaf pine under elevated CO<sub>2</sub> may impact the survival of many of the threatened and endangered species found in these ecosystems (Walker, 1997). Also, given that the small understory species (rattlebox and butterfly weed) did not perform well under CO<sub>2</sub> enrichment, species diversity could be reduced in these communities which may reduce total ecosystem response to elevated CO<sub>2</sub> (Reich et al., 2001). This contention by Reich et al. (2001) is not supported by the current study. Although understory species did not perform well (and could possibly be eliminated) under rising CO<sub>2</sub>, the community as a whole (due to the response of longleaf pine) showed a strong positive response to CO<sub>2</sub> enrichment. In fact, total C content of plants and litter were significantly increased by 3 yr of growth under elevated CO<sub>2</sub> resulting in a gain of 11.4 Mg C ha<sup>-1</sup>. Given that this increased C was largely allocated to woody biomass, the ability of longleaf pine ecosystems to sequester carbon will likely increase as CO<sub>2</sub> rises, assuming the response to elevated CO<sub>2</sub> continues.

## CONCLUSIONS

Individual species within this model community did not always respond in the manner we hypothesized. Perhaps this is not surprising given it has often been noted that response of individual species cannot be reliably predicted—even when based on factors known to influence response to CO<sub>2</sub>—when species are grown in communities. Although rising CO<sub>2</sub> may alter community structure in ways which could impact ecosystem function, productivity and the ability of longleaf pine forests to sequester carbon will likely be enhanced by rising levels of atmospheric CO<sub>2</sub>. It seems apparent that competition among plants confounds our understanding of ecosystem function. This fact highlights the need for additional studies which examine the effects of elevated CO<sub>2</sub> on these processes.

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